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Cloning of the C-Terminal Cytoplasmic Fragment of the Tar Protein and Effects of the Fragment on Chemotaxis of *Escherichia coli*

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A gene encoding only the C-terminal portion of the receptor-transducer protein Tar of *Escherichia coli* was constructed. The gene product was detected and localized in the cytoplasmic fraction of the cell by immunoblotting with anti-Tar antibodies. The C-terminal fragments from wild-type and mutant *tar* genes were characterized in vivo. The C-terminal fragment generated from *tar-526*, a mutation that results in a dominant "tumble" phenotype, was found to be deamidated and methylated by the CheB and CheR proteins, respectively. The C-terminal fragment derived from a wild-type gene was poorly deamidated, and the C-terminal fragment derived from *tar-529*, a dominant mutant with a "smooth swimming" phenotype, was not apparently modified. Cells carrying the C-terminal fragment with the *tar-526* mutation as the sole receptor-transducer protein showed a high frequency of tumbling and chemotaxis responses to changes in intracellular pH. These results suggest that the cytoplasmic C-terminal fragment of Tar retains some of the functions of the whole protein in vivo.

Bacteria sense changes in the chemical composition of their environment through receptors found in both the periplasmic space and the cytoplasmic membrane of the cell. The receptor-transducer proteins (also referred to as methyl-accepting chemotaxis proteins) interact with specific ligands and transduce the ligand-binding event into an internal signal which regulates the direction of bacterial flagellar rotation (for reviews, see references 11, 18, and 23). Increased levels of attractant molecules maintain the counterclockwise rotation of the flagellum and lead to smooth swimming in which the cells do not change direction. Increased concentrations of repellent molecules induce the reversal of direction of flagellar rotation, causing cells to tumble and change their direction of swimming. Thus, modulated changes in flagellar rotation result in chemotaxis towards higher concentrations of attractant molecules and away from repellent molecules.

Four genes that encode transmembrane receptor-transducer proteins in *Escherichia coli* have been identified and extensively characterized (8, 12, 21). Each gene product interacts with a different subset of ligands. The Tar protein modulates the response of the cell to changes in concentrations of dicarboxylic amino acids, including aspartate and glutamate (21). Tar also interacts with the maltose-binding protein as well as with a number of repellents including divalent cations such as nickel (21). The *tsr* gene encodes a transmembrane protein which regulates the responsiveness of the cell to changes in concentrations of the attractant amino acid serine and the repellent hydrophobic amino acid leucine (21). The *tap* gene encodes a transmembrane protein which regulates responses to changes in concentrations of dipeptides (12), while the *trg* gene encodes a protein that is responsible for the ability of the cell to detect changes in concentrations of galactose and ribose (8). In addition to the ligand-binding periplasmic portion of the molecule, each of these proteins also contains a highly homologous cytoplasmic region (2, 3, 10). This domain is associated with two activities: (i) the formation of a signal which can affect flagellar rotation and (ii) the ability of the receptor to respond to a wide range of ligand concentrations. The C-terminal portions of all of the receptor-transducers include two amino acid sequences (the K1 and R1 regions, Fig. 1B) that can be modified in response to extracellular changes in attractant or repellent concentrations (6, 25). Specific glutamines in the K1 and R1 regions are first deamidated, and then the glutamic acid residues are reversibly methylated and demethylated by the products of the *cheB* gene, which encodes a carboxymethylesterase, and the *cheR* gene, which encodes a methyltransferase (6). The steady-state level of receptor-transducer methylation correlates with the extracellular concentrations of specific attractants and repellents (5). Methylation is apparently responsible for the ability of the cell to adapt, i.e., to show behavioral changes over a wide range of attractant and repellent concentrations.

The conclusion that emerges is that all of the receptor-transducer proteins are composed of discrete functional and structural domains (9, 15, 17, 19). One domain primarily formed by the N-terminal portion of the protein is found in the periplasmic space and is responsible for ligand binding. Another subdomain can be mapped to the region of the molecule found in the cytoplasm and may be responsible for the methylation and adaptation functions of the receptor-transducers. In previous experiments (9), we showed that the N-terminal domains of different receptor-transducers could be genetically exchanged to form chimeric receptor-transducers that retained signaling and adaptation functions but responded to the ligand specified by the new N-terminal domain. In biochemical experiments, Mowbray et al. (13) demonstrated that the Tar protein can be separated by proteolysis into two fragments, one corresponding to the N-terminal periplasmic region and the other starting from residue 260 in the Tar protein and corresponding to the C-terminal half of the molecule, including the methylated regions. The N-terminal fragment was still able to bind aspartate; however, the C-terminal fragment was only poorly methylated in vitro. The residual physiological activities of these domains, such as their ability to function in signal transmission or to show adaptation, were not tested. The results, however, suggest that half molecules, i.e., the separated N-terminal or C-terminal domains, might have residual biological activity.

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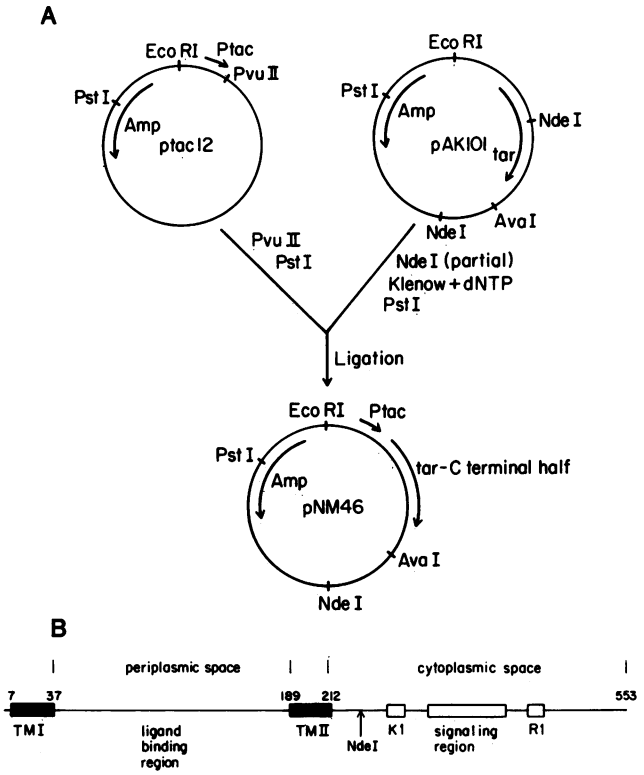

FIG. 1. Construction of a gene encoding the C-terminal fragment of Tar. (A) Procedure used to construct plasmid pNM46 carrying the C-terminal half of the *tar* gene expressed by the *tac* promoter. Plasmid ptac 12 DNA was digested with restriction enzymes *Pvu*II and *Pst*I, and the DNA fragment containing the *tac* promoter region was purified by agarose gel electrophoresis. Plasmid pAK101 DNA was partially digested with *Nde*I, treated with DNA polymerase I large fragment plus dATP and dTTP to make blunt ends, and then digested with *Pst*I. The DNA fragment containing the *tar* gene downstream from *Nde*I was purified by gel electrophoresis. Both DNA fragments were ligated by T4 DNA ligase in the presence of ATP. The resulting plasmid, pNM46, carried the truncated *tar* gene encoding the C-terminal fragment of Tar with the *tac* promoter. dNTP, Deoxynucleoside triphosphate. (B) Structure of the *tar* gene product. The regions TMI and TMII are highly hydrophobic transmembrane segments. The regions K1 and R1 are methylation sites.

Mutational analysis has demonstrated that the periplasmic portion of the receptor-transducer molecule is responsible for ligand interactions and that the cytoplasmic portion is involved in signaling (15, 19). Furthermore, extensive mutagenesis of the C-terminal region resulted in a large number of dominant mutations (15). Some of these showed only smooth swimming behavior, suggesting that the mutation fixed the receptor in a state that could only produce one kind of chemotaxis signal. Another, relatively rare class of dominant mutations fixed the cell in the tumble mode of swimming. These results suggested that the C-terminal domain could be manipulated to signal in a manner independent of the presence of the N-terminal domain. To further explore the activities of the separate domains of the receptor molecules and the effects of specific mutations that might alter the activities of these structural domains, we constructed a gene encoding the cytoplasmic fragment of the Tar protein. The truncated gene can express the C-terminal subdomain of Tar in a cell that has all of the other appropriate receptor-transducer genes deleted. In this report we describe the construction of such a gene and the in vivo assay of the activities of the C-terminal fragment.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* RP437 (wild type for chemotaxis), RP2867 [Δ(*cheR-cheB*)*2241*], RP4968 [Δ(*tap-cheR*)*m58-13*], and RP4971 [Δ*cheBm63-216*] were obtained from J. S. Parkinson (20). KO607 was a *recA56* derivative of HCB429, a strain defective in four receptor-transducers. It carries the alleles Δ*tsr-7021*, Δ(*tar-tap*)*5201*, and Δ*trg-100*, and it was obtained from H. C. Berg (26). RP4372 *recA* was described previously (9).

Plasmid pAK101 was described previously (9). Plasmid ptac12 was obtained from J. Brosius (1).

Chemicals. Restriction enzymes, T4DNA ligase, and the large fragment of DNA polymerase I were purchased from Bethesda Research Laboratories, Inc., Gaithersburg, Md., Boehringer Mannheim Biochemicals, Indianapolis, Ind., and New England BioLabs, Inc., Beverly, Mass., respectively. L-[*methyl*-^{3}H]methionine (15 Ci/mmol) was obtained from ICN Pharmaceuticals Inc., Costa Mesa, Calif. Reagents for immunoblotting were obtained from Bio-Rad Laboratories, Richmond, Calif.

Immunoblotting and *methyl*-^{3}H labeling. Procedures for immunoblotting (15) and *methyl*-^{3}H labeling (9) were described previously. The antiserum used was made by immunization of rabbits with purified Tar (15). This antiserum reacts with the C-terminal portion of both Tar and Tsr (N. Mutoh, unpublished data). Separation of the membrane fraction from the soluble fraction of bacterial cell extract obtained by sonication of whole cells was performed as described previously (17).

Measurement of cell behavior. Cells were grown in tryptone broth consisting of 1% tryptone (Difco Laboratories, Detroit, Mich.), 0.5% NaCl, and 0.5% glycerol at 30°C. At the late log phase of growth, cells were harvested and washed three times with motility medium containing 10 mM potassium phosphate buffer, 10 mM sodium citrate, 0.1 mM potassium EDTA, 1 μM L-methionine, and 0.5% glycerol (pH 6.0). Cells in motility medium were kept at room temperature, and all experiments were performed at room temperature. Measurement of the fraction of smooth-swimming cells was done as described previously (16).

RESULTS

Construction of an expression vector for the C-terminal portion of Tar. Figure 1A outlines the strategy used to prepare a plasmid that would express the C-terminal cytoplasmic region of the Tar protein. We chose to express the C-terminal region starting at the *Nde*I site. This site is conserved in the *tar* and *tsr* receptor-transducer genes, and we have shown previously that a chimeric gene prepared by exchanging the C-terminal ends of the *tar* and *tsr* genes at the *Nde*I site results in a hybrid molecule which is functional (9). This suggests that the C-terminal domain starting from the *Nde*I site includes integral functions. The *Nde*I site is located 50 amino acid residues downstream from the end of the second transmembrane region of the Tar protein; thus, almost the complete C-terminal cytoplasmic region of the molecule is included in the fragment generated by this truncated gene. Furthermore, the sequence at the *Nde*I site includes an ATG codon which could act as the initial codon, in frame, for the synthesis of the C-terminal polypeptide fragment. Coincidently, partial digestion of the Tar protein

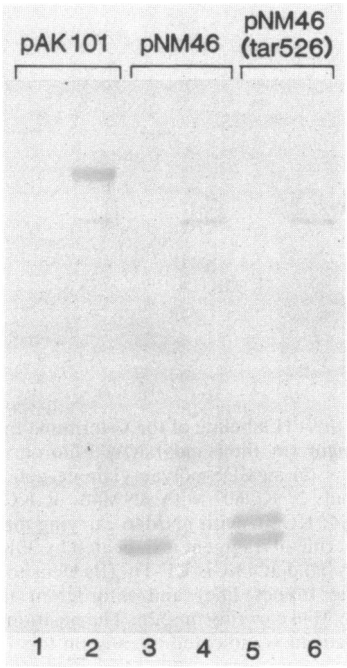

FIG. 2. Detection and localization of the C-terminal fragment of Tar. RP4372 *recA* cells with the indicated plasmid were grown overnight at 30°C in tryptone broth, collected by centrifugation, and suspended in 10 mM Tris buffer (pH 7.6). The cell suspension was sonicated, and the membrane was separated from the soluble fraction by ultracentrifugation. After SDS-polyacrylamide gel electrophoresis, proteins were transferred onto nitrocellulose membranes and stained with anti-Tar antibody. Lanes 1, 3, and 5 show the soluble fraction, and lanes 2, 4, and 6 show the membrane fraction.

by endogenous proteases was found to occur at a position three amino acids downstream from the *Nde*I site (13), suggesting that this C-terminal region starting encodes a structural domain that is resistant to protease digestion. We constructed a plasmid that fused the *tac* promoter and the Shine-Dalgarno sequence from the plasmid ptac 12 (1) to the *Nde*I site (Fig. 1A). The resulting plasmid, pNM46, was introduced into strain RP4372 *recA*. The gene product was easily detected by anti-Tar antiserum after electrophoresis of cell extracts and immunoblotting (Fig. 2).

After preparing this plasmid, we found that the promoter used on pNM46 was a modified form of the original promoter. The sequence had lost the ability to respond to inducers, and it constitutively produced a level of protein which could be estimated by the immunoblotting technique to be approximately the level of Tar normally found in wild-type cells. This fortuitous event allowed us to use the plasmid in studies of in vivo activity, since it produced approximately the normal level of gene product. After disruption of cells by sonication, the membrane and the cytoplasmic fractions were separated, and the intact *tar* gene product was found associated almost entirely with the membrane fraction, while the C-terminal fragment that resulted from the presence of the plasmid was found entirely in the cytoplasmic fraction (Fig. 2). The cytoplasmic fragment had the electrophoretic mobility expected on the basis of the size of a peptide derived from the truncated *tar* gene with a start at the *Nde*I site. The fragment reacted with anti-Tar antibodies and appeared to be relatively stable in the RP4372 *recA* cells.

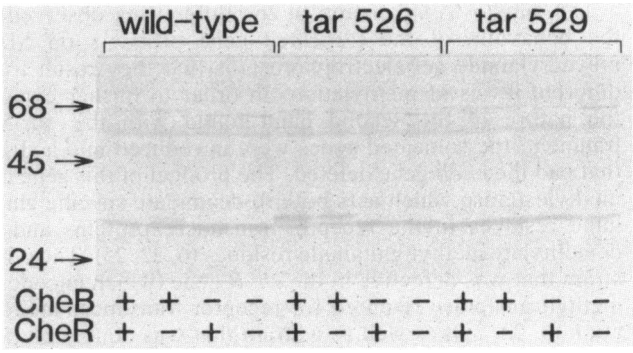

FIG. 3. Modification of the C-terminal fragment of wild-type and mutant Tar protein by methylesterase (CheB) and methyltransferase (CheR). Strains used were RP437 (wild type), RP4968 (*cheR*), RP4971 (*cheB*), and RP2867 (*cheB cheR*). Plasmid pNM46 with the wild-type *tar*, *tar-525*, or *tar-529* allele was transformed into each strain. Cells were grown overnight at 30°C in tryptone broth and mixed with SDS-polyacrylamide gel sample buffer. Proteins were separated by SDS–10% polyacrylamide gel electrophoresis and electroblotted onto a nitrocellulose membrane. Whole Tar protein and the C-terminal fragment were detected with anti-Tar antibodies. Numbers on the left are molecular weights (in thousands).

Characterization of the C-terminal fragment of Tar. In a study of the nature of mutations in the *tar* gene (15), we showed that most of the single-amino-acid changes that resulted in the loss of chemotaxis mapped to the cytoplasmic domain of the Tar molecule in a region including amino acid residues 310 to 475. The DNA sequences of a number of these mutations were determined, and the phenotypes of the mutants with respect to chemotaxis were characterized (15). In order to compare the behavior of the wild-type cytoplasmic Tar fragment with that of some of the mutated fragments, specific mutations were introduced onto plasmid pNM46, and the gene products were examined after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis by immunoblotting (Fig. 2). *tar-526*, a mutation at amino acid residue 436 which converts an alanine to a valine (15), was used. Cells carrying the intact *tar* gene with this mutation show a low level of steady-state receptor-transducer methylation and tumble all of the time (15). Furthermore, the tumbling phenotype is dominant (15); i.e., in the presence of the wild-type allele of another receptor-transducer gene, e.g., *tsr*, the cells show constant tumbling behavior. Thus, the receptor-transducer appears to be fixed in one signaling mode. The effects of this mutation on the cell when the mutation is introduced on the truncated *tar* gene were examined. The Tar-526 C-terminal fragment behaved differently in electrophoresis than did the wild-type C-terminal fragment (Fig. 2); the Tar-526 fragment migrated as two bands on SDS-polyacrylamide gel electrophoresis. We also examined the C-terminal fragment product of another mutation, *tar-529*. This mutation converts a serine at amino acid residue 461 to a leucine (15). When this mutation is present in the complete *tar* gene, it results in a protein which shows high levels of steady-state methylation, and the cell shows constant smooth swimming (15). The *tar-529* mutation is also dominant, i.e., it results in a constant smooth-swimming phenotype even in the presence of a wild-type receptor-transducer gene. The product of the truncated *tar-529* gene, i.e., the Tar-529 cytoplasmic fragment, showed a single polypeptide band after SDS-polyacrylamide gel electrophoresis (Fig. 3).

The simplest explanation of the differences observed in the behaviors of the truncated gene products on SDS-polyacrylamide gel electrophoresis is that they result from different levels of methylation. In order to further explore the nature of the second band found with the Tar-526 fragment, the truncated genes were introduced into a strain that had the *cheB* gene deleted. The product of this gene is a methylesterase which acts both to deamidate specific glutamine residues in the receptor-transducer proteins and to demethylate methyl-glutamate residues (6, 22, 25). The other strain that was defective in the *cheR* gene (which encodes a methyltransferase required for receptor-transducer methylation [6, 22, 25]) as well as a strain that was doubly mutant in *cheR* and *cheB* were also used as hosts for the fragments. Figure 3 shows the results of SDS-polyacrylamide gel electrophoresis followed by immunoblotting with anti-Tar antiserum with the wild-type and mutant gene fragments in a variety of backgrounds deficient in methyltransferase and methylesterase activities. A predominant rapidly migrating band and very low levels of a more slowly migrating band were found with the wild-type fragment. However, in strains that lack the *cheB* gene, only the rapidly migrating band was found. The C-terminal fragment product of the *tar-526* gene also showed two distinct bands with mobilities similar to those seen with the wild-type fragment. The upper band was absent in strains that lack the *cheB* gene product. On the other hand, the truncated *tar-529* gene showed only a single product under all conditions, independent of the presence of the *cheB* or *cheR* gene product. In the case of whole Tar protein (22), the deamidated form was found to migrate more slowly than the unmodified molecule. We interpret the two bands found with the truncated receptor-transducers as corresponding to the nascent and deamidated forms of the C-terminal fragment. Thus, these results suggest that both the wild-type and the *tar-526* gene products are capable of being modified by the CheB deamidase. We suggest that the wild-type fragment shows a low level of residual deamidated product, while the Tar-526 fragment shows high levels of the deamidated fragment. The Tar-529 fragment may not be able to undergo deamidation, and the position of the band therefore remains the same.

In order to explore the question of the degree of methylation of the cytoplasmic fragments, the plasmids were introduced into strain KO607. This strain lacks the known four receptor-transducer genes; however, all of the other chemotaxis genes are present and intact. For this strain, steady-state levels of methylation were measured by incubating the cells with L-[*methyl*-^{3}H]methionine (Fig. 4). Only the Tar-526 fragment showed high levels of methylation that were easily detectable (Fig. 4A). The wild-type fragment and the Tar-529 fragment did not show clearly discernible methylated bands (Fig. 4A). In this system, the methylated band comigrated with the lower band found in the Tar-526 background (Fig. 4B). These data suggest that the slower-migrating form of the fragment corresponds to the deamidated molecule which is present in the CheB-containing cells, while the lower band includes two species, one corresponding to the fragment before deamidation and the other corresponding to the deamidated and methylated gene products. Further resolution of these forms has not yet been obtained by changing the conditions of the polyacrylamide gel electrophoresis.

Behavioral effects of cytoplasmic receptor-transducer fragment mutants. The *tar* mutants that lost the ability to swarm on tryptone plates were initially classified into six groups based on the steady-state level of methylation of the Tar protein and on their swimming behavior (15). Thus, when the intact *tar-526* gene was introduced into KO607, the cells showed extreme tumbling bias (the smooth-swimming fraction was less than 10% of the total) and did not show any chemotaxis toward L-aspartate (Fig. 5B). Furthermore, the methylation pattern of the mutant protein was not changed after addition of 10 mM α-methylaspartate (a nonmetabolizable analog of aspartate), while the wild-type protein showed highly methylated bands after addition of α-methylaspartate (Fig. 6). We wanted to compare the behavior of cells carrying the fragment with that of cells with the whole mutant receptor-transducer. Since the fragments lacked ligand-binding components, we could not test for their activ-

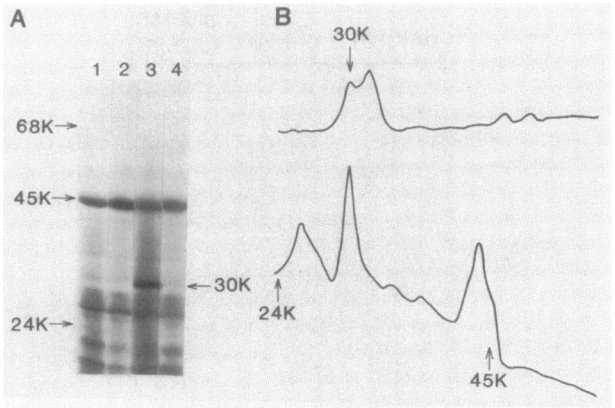

FIG. 4. *methyl*-^{3}H labeling of the C-terminal fragment of Tar. K, Molecular weight (in thousands). (A) Fluorogram of *methyl*-^{3}H-labeled proteins on an SDS-polyacrylamide gel. Lanes: 1, KO607 without plasmid; 2, KO607 with pNM46; 3, KO607 with pNM46 carrying *tar-526*; KO607 with pNM46 carrying *tar-529*. The *methyl*-^{3}H-labeled C-terminal fragment is indicated by 30K on the right. The labeled protein band at 43K is EF-Tu. (B) Densitometric patterns of immunoblotting (upper line) and fluorogram (lower line) from KO607 with pNM46 carrying *tar-526*. The position of the methylated C-terminal fragment is indicated by 30K on top.

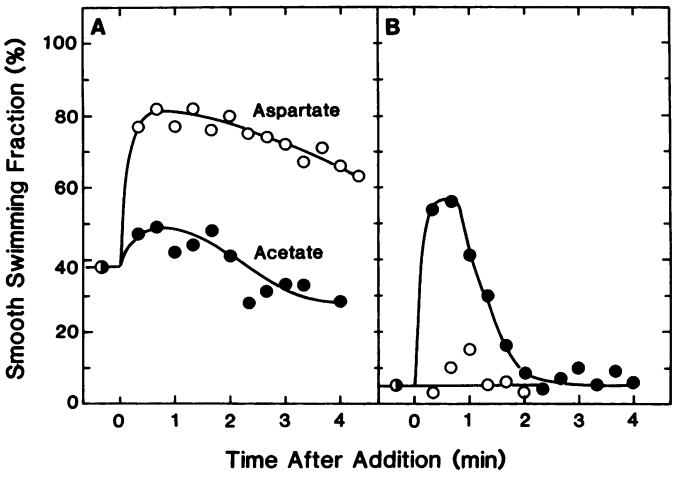

FIG. 5. Effects of aspartate and internal pH on swimming behavior of cells with wild-type and mutant whole Tar. Cells in motility medium at room temperature were mixed with 10 μM sodium L-aspartate or 30 mM sodium acetate (pH 6.0), and the fraction of the smooth-swimming cells was measured. (A) KO607 lysogenized with lambda carrying wild-type whole *tar*. (B) KO607 lysogenized with lambda carrying whole *tar-526*.

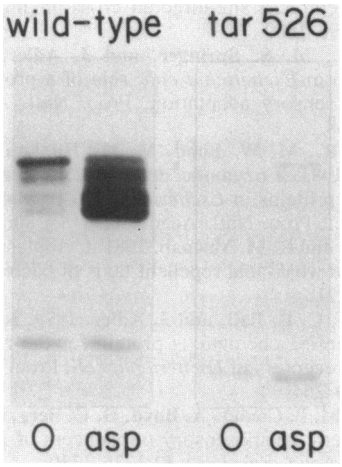

FIG. 6. Effect of α-methylaspartate on steady-state level of methylation of mutant whole Tar protein. RP4372 *recA* cells carrying pAK101 with or without the *tar-526* mutation were labeled with L-[*methyl*-^{3}H]methionine (15 Ci/mmol) for 50 min at 30°C. α-Methylaspartate (10 mM) was added (lanes asp) 40 min after addition of L-[*methyl*-^{3}H]methionine. Lanes 0 received no α-methylaspartate. After separation of proteins on an SDS-polyacrylamide gel, *methyl*-^{3}H-labeled proteins were visualized by fluorography. The labeled band on the bottom in each lane is EF-Tu protein.

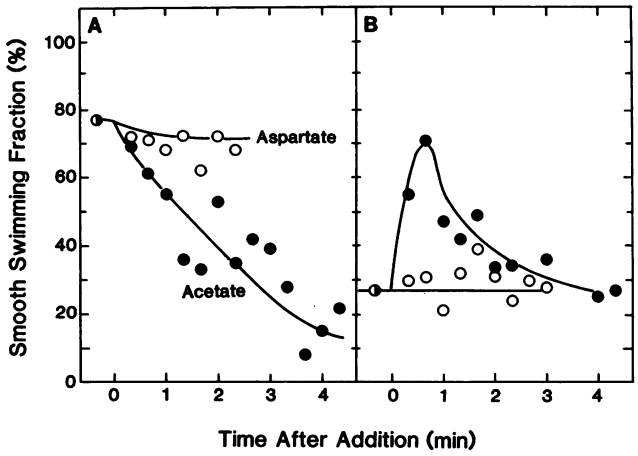

FIG. 7. Effect of changes in internal pH on swimming behavior of cells carrying wild-type and mutant C-terminal fragments. (A) KO607 with pNM46. (B) KO607 with pNM46 carrying *tar-526*.

ities by using attractant ligands such as aspartate. However, they may retain some responsiveness, and residual activity could be tested for by lowering the intracellular pH by the addition of weak acids; this has an effect on cell behavior which is manifested through the cytoplasmic region of the receptor-transducer proteins (7, 24). Lower intracellular pH causes a tumbling response when it is detected through the Tsr protein, and it induces a smooth-swimming response when detected by the Tar protein (7, 9, 24). To test the ability of the intact mutated *tar* gene product to respond to cytoplasmic and periplasmic stimuli, cells were lysogenized with bacteriophage lambda carrying *tar-526* in order to introduce a single copy of the mutated receptor-transducer gene. Cells so treated showed a marked tumble bias (Fig. 5B). When the cells were stimulated with 30 mM sodium acetate at pH 6.0, they responded in a transient manner by increasing the number of cells swimming smoothly, and after a few minutes they returned to the tumble mode of swimming (Fig. 5B). These results suggest that the mutation from alanine to valine at amino acid residue 436 in the intact receptor-transducer may have eliminated the function required for transduction of external signals (e.g., the response to aspartate) but not for internal stimulation such as changes in internal pH.

Based upon the results of experiments involving measurement of chemotaxis after intracellular pH changes and from the behavioral responses induced by chimeric proteins made from elements of Tar and Tsr (9), it has been suggested that the domain of Tar that senses lower internal pH as an attractant is located in the region between the *Nde*I and *Cla*I sites on the *tar* gene. We attempted similar experiments with cells that have only the C-terminal fragment. Cells carrying the Tar-526 fragment showed predominant tumbling behavior similar to that of cells carrying the whole protein with the same mutation (Fig. 7B). When acetate was added, these cells showed transient attractant responses to the lower internal pH. On the other hand, cells which had the wild-type Tar fragment were primarily smooth swimming, and therefore it was difficult to detect a response to changes in internal pH. The only response measured when acetate levels were increased in the presence of the wild-type C-terminal fragment was a slow drift of the population to the tumble mode of swimming. A transient attractant response to acetate may have been obscured, since the initial state of the population was already highly biased toward smooth swimming.

DISCUSSION

The C-terminal cytoplasmic fragment of the receptor-transducer protein Tar was obtained by cloning the *tar* gene downstream of the *Nde*I site into plasmid ptac 12. The fragment was detected by immunoblotting of SDS-polyacrylamide gels with anti-Tar antiserum, and it has an apparent molecular weight of 30,000. Separation of the membrane fraction from the soluble fraction revealed that the fragment was a soluble protein localized in the cytoplasm.

It has been reported that receptor-transducer proteins include domains involved in ligand binding, transmembrane signaling, intracellular signaling, and adaptation (15, 17, 19). Purification and characterization of the C-terminal fragment of Tar, which was obtained as a cleaved product from the whole protein by endogenous protease, revealed that it was methylated poorly in vitro (13). The C-terminal fragment produced by cloning the gene differed from the proteolytically derived C-terminal fragment by three additional amino acid residues (methionine, glutamine, and arginine) at the N terminus, and the in vivo behavior of this fragment derived from the wild-type and mutant *tar* genes was studied. A mutant C-terminal fragment (the Tar-526 fragment) was found to be modified and methylated by CheB and CheR proteins in vivo. The wild-type C-terminal fragment was also modified, but only to a small extent. We have no evidence for modification of the Tar-529 fragment. These results suggest that mutations in the C-terminal region affect the conformation of the fragment and control its efficacy as a substrate for the modifying enzymes.

While the wild-type and mutant cytoplasmic fragments of Tar have lost their ability to generate responses to attractants such as aspartate, they do retain some signaling ability. Thus, in the absence of other receptor-transducers, the

Tar-526 fragment was able to bias the behavior of the cells so that they were primarily in the tumble mode. This fragment also generated an attractant response to transient changes in intracellular pH. These results suggest that some forms of the C-terminal cytoplasmic fragment retain a semblance of their biological activity. These soluble fragments may be very useful in generating reconstituted chemotaxis signaling systems in vitro.

It is difficult to directly compare the function of the cytoplasmic fragment with that of the intact transmembrane protein. Thus, for example, the Tar-526 fragment was readily modified and showed methylation (Fig. 4), while its intact counterpart was generally found to show a very low level of steady-state methylation (Fig. 6). On the other hand, the wild-type and Tar-529 intact proteins were readily methylated, but their C-terminal fragments showed little methylation. The simplest explanation is that the presence of the periplasmic and transmembrane domains strongly influences the configuration of the C-terminal domain in both the signaling and nonsignaling states. Our previous work on suppressors of mutations in the N-terminal transmembrane region of Tar (17) suggested that N-terminal and C-terminal regions of the molecule may interact indirectly and that the state of the N-terminal portion of the molecule may stabilize the structure of the C-terminal portion. Thus, in the absence of the N-terminal portion, the properties of the C-terminal fragment are altered. Nonetheless, the mutant fragments provide a useful model of the function of the intact protein. In studies of receptor-transducer methylation and modification, Mowbray and Koshland (14) and Falke and Koshland (4) have concluded that there are conformational changes in the structure of the receptor-transducer that are affected by methylation and that a dynamic relationship exists between the conformations of the periplasmic and cytoplasmic portions of the molecule. The activities of the cytoplasmic C-terminal fragments suggest that the periplasmic region of the molecule acts as a regulatory domain modulating the ability of the C-terminal polypeptide to generate signals. The activity of the C-terminal domain may depend upon its specific conformation. Mutations in this region could act to stabilize one of a number of possible conformers, thus preventing the receptor-transducer from cycling. The soluble C-terminal fragments may allow us to biochemically define the various conformational and functional forms of the C-terminal region of the receptor-transducer.

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